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Issued November 6, 1912.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF SOILS—BULLETIN No. 89.
MILTON WHITNEY, Chief.

THE CHEMISTRY OF STEAM-HEATED SOILS.

BY

OSWALD SCHREINER,
Scientist in Charge of Fertility Investigations,

AND

ELBERT C. LATHROP,
Scientist in Fertility Investigations.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Soils,
Washington, D. C., June 7, 1912.

Sir: I have the honor to transmit herewith the manuscript of a scientific paper entitled "The Chemistry of Steam-Heated Soils," by Dr. Oswald Schreiner, in charge of Soil Fertility Investigations, and Mr. Elbert C. Lathrop, scientist in Soil Fertility Investigations, of this bureau.

The results set forth therein are of fundamental importance in connection with biochemical processes in soils, and I therefore recommend its publication as Bulletin No. 89 of the Bureau of Soils.

Respectfully,

Milton Whitney,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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THE CHEMISTRY OF STEAM-HEATED SOILS.

INTRODUCTION.

The subject of the effect which heat has upon soils is rather prominently before agricultural investigators at the present time. This subject has received increasing attention during the last decade, particularly from the point of view of sterilization and partial sterilization. There has been much valuable information obtained along the biological lines concerned; also much speculation in this regard without due consideration of the chemical and physical changes involved, owing largely to the fact that these were not known or but imperfectly understood. The biological factors can not and should not be ignored, but it must be remembered that they are only of significance or interest in the light of the biochemical changes which they produce in the soil. Moreover, any biochemical changes produced in the soil through other means, be they cultural or by the addition of manures and fertilizers or by the influence of steam heating, in turn affect the biological activities. The chemistry, therefore, of the soil both before and after heating in sterilization work becomes of the greatest significance to the biological worker.

Since heat activates the changes going on normally in soils, it is obvious that a study of heated soils also throws light upon the biochemical changes taking place in soils under field conditions. The present bulletin is a contribution to the knowledge concerning soil organic matter and the changes which it undergoes. It is hoped that the results set forth will be of interest and value alike to the soil biologist and the practical worker with sterilized soils in greenhouse or field, as well as to the soil investigator dealing with the subject of the soil's fertility and infertility.

THE EFFECT OF HEATING ON SOILS.

Until the investigations of Franke[1] in 1888 soil investigators did not consider that the process of sterilization of soils by heat effected any changes in either the mechanical nature or chemical composition of the soils so treated. In attempting to explain the increased yield observed on sterilized soils he decided that the steam heating of the soil had caused some chemical changes. He found that the heated

[1] Ber. bot. Ges., 6, lxxxvii (1888).

soils contained a great deal more soluble matter than the unheated soils; the moor soils contained more than twice as much and the sandy soils not quite twice as much. The increased productiveness was, therefore, to be explained by the fact that by the process of sterilization more material was rendered soluble and made available for plant use. Schmoeger[1] found that from a soil which had been heated in an autoclave at 140° to 160° C. for several hours he could extract with 12 per cent hydrochloric acid almost as much phosphate as was obtained from the ash of the soil, and much more than could be obtained in the same way from the soil unheated. He concluded that a part of the phosphoric acid existed in the soil in the form of lecithins or nucleic acids. Liebscher[2] at the same time found that by steaming the soil there was an increase in soluble phosphate and also an increase in soluble nitrogenous compounds, which thus became available to plants. According to him, steam sterilizing may be viewed as nitrogen fertilizing.

Deherain and Demoussy[3] found that the production of carbon dioxide obtained by heating soils increased in a measure proportional to the temperature until 110° is reached. The quantity obtained they considered higher than could be attributed to bacterial action. At a temperature of 120° decomposition is produced by oxidation and reduction, it being found that some soils at this temperature absorbed more oxygen than was given off as carbon dioxide. By sterilizing the soil they observed an increased ammonia-nitrogen content and were able to prevent nitrification, although the sterilized soil when inoculated with a portion of the unsterilized soil produced larger quantities of nitric nitrogen than did the unsterilized soil.

Pfeiffer and Franke[4] confirmed Liebscher's view that there is an increase in assimilable nitrogen in the heated soil, and showed large increases both in crop yield and in nitrogen assimilated from the heated soil as compared with the unheated soil. Richter[5] heated a garden soil and found that the heating had increased the absorptive power of the soil for water and the amount of easily assimilable nitrogenous compounds; the total water-soluble organic matter had been increased almost threefold.

The first account of the bad effects of soil sterilization on plant growth is given by Dietrich,[6] who states that, although there is an increase in soluble nutrients, especially nitrogenous compounds, in the heated soils, there is also a poison formed by the alteration of the organic matter of the soil Thus the plants are influenced by two

[1] Ber. deutsch. chem. Ges., **26**, 386 (1893).
[2] Deutsch. Landw. Presse, **20**, 976 (1893).
[3] Ann. agron., **22**, 305, (1896).
[4] Landw. Vers.-Stat., **46**, 117 (1896).
[5] Landw. Vers.-Stat., **47**, 269 (1896).
[6] Jahresb. Landw. Vers., Marburg, 1901–2; Bied. Centrlb., **32**, 68 (1903).

factors in the heated soil which are opposed to each other, one the more available plant nutrients, and the other the poisons arising during and following the sterilization. According to the amount of this poison and the sensitiveness of the plant to it, the one or the other factor predominates, and where the plants were less susceptible to the poison there was observed an increased absorption of soluble nitrogen by the plants. The addition of calcium carbonate to the soil to be sterilized prevented or almost prevented the poisonous action. Schulze[1] also noticed the harmful effect, and he says in this regard that not only in the physiological properties, the killing of bacteria, but also in the physical chemical properties of the soils changes are produced by sterilization. The difficultly soluble material of soil, mostly of humus nature, is thus made more soluble, and this soluble material has a harmful effect on the growing plants, but this effect varies with different soils. He further states that dry weight of the plants grown on the sterilized soil was as low as 26 per cent of the dry weight of those grown on the unsterilized soil. The effect of this injurious substance does not last through the whole life of the plant, for as the plants grow older they become more vigorous on the sterilized soils. Koch and Luken[2] found that with soils which had received applications of fertilizers in each case the heating produced immediate injurious effects, though these were found to be unimportant if the plants were started in the summer instead of in the spring.

Darbishire and Russell[3] in 1907 made a study of the effects of partial sterilization on soils, using for the purpose of sterilization volatile antiseptics and steaming at 95° and 120° C. They found that the crops could get from the heated soils three times as much nitrogen and twice as much phosphoric acid and potash as from the unheated soils and that the increase in dry matter in the plants was very considerable. Analysis showed an increase in the amount of nitrogen, phosphoric acid, and potash taken up by the crop and in the percentage of nitrogen and phosphoric acid in the dry matter. They believed that the increased availability of the plant nutrients is connected with the modification of the bacterial flora, brought about by partial sterilization, although when the soils are sterilized by heat a certain amount of decomposition also takes place.

Two years later Russell and Hutchinson[4] report further experiments along this line. They believe that the effect of partial sterilization on soils is to destroy the larger phagocytic microorganisms which inhibit the growth of the beneficial bacteria. They state that

[1] Jahresb. Vertreter angew. Bot., 1, 37 (1903); Centrlb. Bakt. Par., 2, abt. ii, 716 (1903).
[2] Jour. f. Landw., 55, 161 (1907).
[3] Jour. Agr. Sci., 2, 305 (1907).
[4] Jour. Agr. Sci., 3, 111 (1909).

the increased productiveness of partially sterilized soils is due to an increase in the quantity of ammonia present, and that this increase is the result of an increased decomposition of soil substances by bacteria. When the soil was heated to 95°, there was a small immediate production of ammonia and little change took place for a few days; this period of inaction was followed by one of rapid change, during which ammonia was produced in considerable quantities; lastly, a slow period of change set in and the further production of ammonia was small. Soil heated to 125°, at which temperature all bacteria are killed, behaved very differently; after the first production of ammonia by heating there was no further change. Heating the soil rendered it unfit for the development of the nitrifying bacteria; apparently during the heating a compound was formed which was toxic to them and not to the ones producing ammonia. However, by the time the second crop was growing on the heated soil the nitrifying bacteria added developed abundantly, suggesting that the toxic body slowly disappears from the soil. In their relation to plant growth the heated soils were peculiar in that they supplied not nitrates but other nitrogenous compounds to the plants. The greater activity of nitrifying than of ammonifying bacteria in normal soils they attribute to the presence of the larger organisms, particularly protozoa, which through their destruction of the ammonifying bacteria constitute a limiting factor in soil fertility.

At about the same time Pickering[1] conducted experiments in regard to germination and plant growth on sterilized soils, which may be summarized as follows: Soils which have been heated to temperatures from 60° to 150° C. behave unfavorably to the germination of seeds, the total number of seeds germinating decreasing in most cases, and the time necessary for their germination increasing with the temperature of heating. According to Pickering, the retarding effect on germination can not be explained by an alteration in the bacterial condition of the soil, for the alteration extends progressively at temperatures beyond that sufficient to kill all bacteria, and, moreover, no similar results were obtained with sand, even when it had been thoroughly impregnated with soil bacteria to begin with. By heating the soil an increase of soluble constituents occurs. There is an increase in mineral matter, but especially in organic and nitrogenous matter; the increase in the latter has been found to be proportional to the temperature of heating and to the increase in the time required for germination. The increase in the time of germination appears to be due, he concludes, to the formation of a nitrogenous compound in the soil which is inhibitory to germination. This compound was sufficiently stable in solution for an extract of a heated soil to affect a normal soil when added to the latter; it also does not

[1] Jour. Agr. Sci., 2, 411 (1908); Ibid., 3, 32 (1908).

seem to be destroyed when the soil containing it is kept at a low temperature for several months, but at higher temperatures and in the presence of moisture it generally loses its inhibitory properties, probably through oxidation. This substance is not an acid, for the soils were heated in the presence of an excess of chalk and no acids were formed. The production of the substance inhibitory to germination seems to be a property common to many soils. The substance which is toxic to germination is not necessarily so to plant growth, or it may be destroyed before growth becomes active. The heated soils behaved toward the starting of the growth of young apple trees as it did toward the germination of seeds, the starting of the growth being considerably delayed; later, however, the growth of the trees became very vigorous.

Seaver [1] and Seaver and Clark [2] studied the effect of heated soil on certain fungi, *Pyronema omphalodes*, which apparently developed only on heated or burnt soils.

From a study of the effects of steam sterilization on the water-soluble matter in soils, Lyon and Bizzel [3] conclude that by the steaming of soils substances injurious to plant growth arise. The growth of wheat plants on steamed soil served to hasten its recovery from the injurious effects of heating, and the time required for the various soils to recover from these effects was, with one exception, in the order of their relative productiveness. The steaming greatly increased the soluble nitrogenous compounds and phosphoric acid; the nitrates were reduced to nitrites and to ammonia, but most of the ammonia was formed from organic matter. On standing, without plants growing upon them, the steamed soils gradually decreased in their content of soluble matter, including ammonia and nitrogenous organic matter. If, however, the steamed soils were planted, they showed much more soluble matter at the end of three months than did the unplanted, steamed soils.

In greenhouse work, particularly for the forcing of plants, steam sterilization of the soil is largely used. Stuart,[4] Stone and Monahan,[5] and Stone and Smith [6] report experiments along this line. They find that in the forcing of plants, particularly lettuce and cucumbers, soil sterilization is necessary. The sterilization has the effect of killing the nematodes and lessening the amount of infection, especially *Rhizoctonia* in lettuce. It also increases the growth of the plant, as high as 33 per cent increased yield having been obtained by such procedure. For the purpose of sterilizing large beds of soil, various

[1] Mycologia, 1, 131 (1909).
[2] Ibid., 2, 109 (1910).
[3] Cornell Agr. Expt. Sta. Bul. 275 (1910).
[4] Vermont Expt. Sta., Ann. Report, 1905, p. 297.
[5] Mass. Expt. Sta., Report, 1904.
[6] Mass. Expt. Sta., Report, 1902, p. 38; Ibid., 1901, p. 57.

forms of apparatus have been designed and are reported by Rudd,[1] May,[2] Selby,[3] and the experimenters mentioned above.

It is, however, not the purpose of this bulletin to enter into a discussion of such applications, but to confine itself to the biochemical changes involved in the heating incident to sterilization.

Notwithstanding the fact that some of the results obtained by the investigators mentioned in the foregoing review, as well as some others, are contradictory, especially in regard to the effects of steam sterilization on crops, they seem to warrant the following conclusions:

By steam heating the physical, chemical, and physiological properties of the soils are more or less altered. The chemical changes consist in the increase in soluble matter in the heated soils. This is partially of an inorganic nature, potash and phosphoric acid being mainly determined, but the largest increase is in the organic matter rendered soluble. Ammonia may be formed by the reduction of the nitrates to nitrites and ammonia, but is especially formed from the decomposition of organic matter. Large amounts of nitrogenous matter are made soluble and apparently more available for plant use, and carbon dioxide is produced in large quantities.

By partial sterilization the larger phagocytic microorganisms, which are said to limit the activity of ammonifying bacteria in normal soils, are killed. The nitrifying bacteria do not grow on the freshly sterilized soils, there apparently being compounds formed which are of an inhibitory nature, but which disappear after a time. By complete sterilization all life in the soil is killed and reinoculation must be resorted to.

With regard to crop production in general, the sterilized soils seem to contain some substance which is decidedly injurious both to germination and to plant growth, particularly if calcium carbonate is not present in excess during the heating to prevent the forming of acids. After standing, and especially after planting for some time, these injurious effects weaken and finally disappear, with the result that the growth on the sterilized soil is greater than that on the normal soil, the plants showing large increases in dry weight and in the amounts of plant nutrients absorbed by them.

SCOPE OF THE INVESTIGATION.

In the foregoing enough of the early work has been presented to show that chemical changes occur in soils when heated as in the process of sterilization usually employed. The nature of the changes are not made clear, although a number of suggestions or hypotheses have been put forward. These concern themselves partly with the

[1] Am. Florist, 9, 171 (1893)
[2] Ibid., 13, 721 (1898).
[3] Ohio Expt. Sta., Bul. 73, 231 (1896).

explanation of the increase in mineral constituents, due to altered absorption properties for these constituents or brought about by the greater hydrolysis or solubility of the minerals under the conditions of higher temperature. Nearly all of the investigators have particularly noticed the increase in soluble nitrogen and coincident with this the increase in soluble organic matter. It is these latter considerations that have been the source of most of the speculations to explain the increase and decrease, as the case may be, of plant growth on soils thus heated. These views are usually coupled with the biological idea which furnished the motive for the experiment.

In no case has any definite compound been obtained, either as an end or intermediary product of the changes brought about by the heat, and the subject is left in the same state of indefinite generalization in which soil organic matter or humus found itself during the course of the last half century. In view of this lack of knowledge concerning the chemistry of soil organic matter itself, and its transformations under ordinary conditions, it is not to be expected that changes like the above could be clearly defined. As a consequence of this lack of knowledge concerning soil organic matter, these investigations had to confine themselves to the use of simple analytical methods, such as the determination of ammonia formed, the determination of the quantity of organic matter soluble in water or dilute acids, and the amount of nitrogen in organic combination contained therein. In addition to the above, there was also usually made a determination of total soluble solids and of phosphoric acid and potash, the other principal plant nutrients, besides the nitrogen.

The investigations of the last few years in this laboratory have brought with them an increasing knowledge of the nature of organic matter, especially with regard to the isolation and identification of specific chemical compounds from soils, so that the biochemical changes taking place in soil are becoming clear. In connection with these investigations methods for the recognition or isolation of specific chemical compounds have been developed. With such knowledge at hand, it seemed possible to go one step further and show the changes produced in soil organic matter in the process of sterilization by heat, and thus to be able to interpret the nature of such changes. By such an investigation, specific and definite knowledge concerning the organic constituents which actually exist in the heated soil and affect plant growth favorably or unfavorably, as the case may be, can be determined, and the nature of the interactions of such compounds in soil solutions interpreted. In the present bulletin are presented the results obtained along this line, which tend to throw a different light upon the subject and, moreover, to make clearer the changes which slowly take place in soils under normal conditions.

THE SOILS STUDIED.

The soils used in this investigation are two types of the Coastal Plains Province known as Elkton silt loam and Sassafras silt loam.[1] These two soils were collected from the same farm, about 4 miles east of Easton, Md., April 28, 1911. The samples taken were from several spots within a radius of 40 feet and were within 150 yards of each other, in adjoining fields.

Although these soils have the same geological origin, they differ rather widely in physical and chemical characteristics and in fertility. The largest areas of the Elkton soil are found in the peninsulas lying between estuaries. The central parts of most of these necks are nearly level, and the sluggish surface drainage, together with the character of the material, accounts for the formation of this type. Smaller areas are found in the uplands wherever similar conditions prevail.

The Elkton silt loam is associated with the Sassafras silt loam and is derived from the same kind of material. The differentiation is due mainly to drainage conditions. While the two soils are very distinct in color and in general agricultural value, the change from one to the other is frequently so gradual that it is difficult to draw an exact boundary. The samples taken are strictly typical.

ELKTON SILT LOAM.

The Elkton soil is a light-gray to almost white silt loam containing 82 per cent silt, 10.5 per cent clay, about 5.5 per cent fine sand, and practically no coarse sand. When in good tilth it is loose and floury, but when wet it has a tendency to run together and on drying to form a smooth hard surface, with minute cavities interspersed through it. The subsoil forms a compact stratum, very hard when dry, and when wet somewhat more plastic or sticky than the surface soil. Along the line of contact with rootlets there is a tendency to show a reddish-brown coloration, probably because of the oxidation of iron compounds by the roots. In places pieces of unrotted manure and plant residues were found, some of them retaining almost their original structure. Clover does not grow well on this soil, nor is it a good soil for corn, for the land is too cold and wet to be adapted to the latter crop. Wheat, however, is grown on it and compares rather favorably in yield with that grown on the Sassafras soil. The soil is acid and would be benefited by applications of lime and manure and by improvement in drainage conditions. However, the frequently unsatisfactory yields from well-drained lands indicate a

[1] Soil Survey of the Easton Area, Maryland. By H. H. Bennett, W. E. Tharp, W. S. Lyman, and H. L. Westover. Bureau of Soils, U. S. Dept. Agr. (1909).

poor structural condition and probably the presence of substances injurious to plant growth.[1]

SASSAFRAS SILT LOAM.

The Sassafras soil to a depth of 8 or 10 inches is, when moist, a friable yellow silt loam. It contains 72 per cent silt, 14.2 per cent clay, quite a little fine sand and some coarse sand, yields well to tillage, and the cultivated land has a soft loamy surface. The subsoil contains more clay than the soil and is rather compact, though between the depth of 15 to 30 inches it is somewhat granular. The drainage is good and the sandy substratum gives excellent underdrainage and prevents any undue accumulation of water where the surface is slightly depressed. The soil is well adapted to corn, wheat, hay, and clover.

SOME ANALYTICAL DATA.

For this investigation 1,000 pounds of each of the soils were collected. The Elkton soil was in clover, following wheat the year before, which had followed corn. The corn had yielded about 15 bushels to the acre, wheat about 20 bushels, and the clover was very poor. The soil was very moist and plastic and was collected from several spots upon which nothing was growing. The field was full of such spots. The Sassafras soil was collected in the next field, which had been plowed but not harrowed, and the soil was in excellent condition. Corn the preceding year had yielded 50 bushels, and the year before wheat had given about 20 bushels to the acre. The soils were sifted to remove any large stones or organic débris and then well mixed by repeated shoveling. The mechanical analyses were as follows:

TABLE I.—*Mechanical analyses.*

Soil type.	Fine gravel 2–1 mm.	Coarse sand 1–0.5 mm.	Medium sand 0.5–0.25 mm.	Fine sand 0.25–0.1 mm.	Very fine sand 0.1–0.05 mm.	Silt 0.05–0.005 mm.	Clay 0.005–0.00 mm.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Elkton silt loam	0.2	0.8	0.9	1.4	4.2	82.0	10.5
Sassafras silt loam	0.3	2.1	1.8	1.6	7.6	72.2	14.2

Analysis shows that these two samples contain practically the same quantity or organic matter, the Elkton 2.25 per cent and the Sassafras 2.24 per cent, the former contains 0.13 per cent of carbonates as carbon dioxide, and the latter only 0.03 per cent. The soils are both acid to litmus paper, the Sassafras being slightly the more acid of the two. In regard to their oxidizing and catalyzing

[1] Examination of Soils for Organic Constituents, Especially Dihydroxystearic Acid, by Oswald Schreiner and Elbert C. Lathrop. Bul. 80. Bureau of Soils, U. S. Dept. Agr. (1911).

power, that is, the decomposition of hydrogen peroxide, both are very weak, the Elkton showing none at all and the Sassafras only a little. They both contain practically the same percentage of total nitrogen. When the soils are boiled with hydrochloric acid and the different forms of nitrogen determined according to the method of Osborne and Harris[1], which was applied to soils by Shorey,[2] the soils show only trifling differences.

TABLE II.—*Different forms of nitrogen in Elkton silt loam and Sassafras silt loam.*

[Results expressed in per cents of total nitrogen in oven-dried soil.]

Form of nitrogen.	Elkton.	Sassafras.
	Per cent.	*Per cent.*
Per cent of total N in soils	0.0859	0.0869
N insoluble in HCl	8.17	8.92
N in HCl solution	91.83	91.08
N as NH_3 in solution	0.83	2.27
N as amides in solution	12.06	12.45
N as "humin" N in solution	18.96	19.63
N as diamino acids in solution	5.53	7.17
N as mono-amino acids in solution	54.45	49.56

From the table it is apparent that the only difference worthy of mention is the fact that the Sassafras soil contains slightly more ammonia than does the Elkton soil, but this is much too small to account for the existing differences in fertility.

From the foregoing analytical data it is apparent that, in a general way, these two soils are very similar in their organic composition and that no methods of analysis, excepting the actual isolation of the various definite organic compounds which compose the organic matter, will show wherein the difference in their organic composition lies.

The methods of farm practice have been identical with these soils, and each has received the same kind of débris, manures, plant residues, etc., which by processes of decomposition form the organic matter of soils. If differences in the organic matter of these soils are shown to occur, they are due to different processes of decomposition taking place in the soils.

COMPARATIVE STUDY OF HEATED AND UNHEATED SOILS.

It is obvious that such an investigation as the present one derives its chief value from the fact that it is comparative for the heated and unheated soil of the same type. To attain this end, it is therefore necessary to know as much as possible concerning the nature of the organic matter in the normal or unheated soil so

[1] Jour. Am. Chem. Soc., 25, 323 (1903).

[2] Agr. Invest. Hawaii in 1905, Rpt. Chemist., p. 34; see also Lathrop and Brown, Jour. Ind. Eng. Chem. 3, 657 (1911).

that the changes themselves, which take place as the result of heating, can be ascertained by direct comparison with the unheated soil as the control. While some analytical data have been obtained with these soils, the chief time and energy have been expended in the actual isolation or determination of compounds of definite chemical identity.

METHOD OF HEATING.

For the purpose of heating the soils an autoclave, such as is in general use, was employed. The autoclave is of copper, so constructed that it may be automatically kept at two atmospheres of steam pressure for a given length of time. The soils were heated in 1-gallon stoneware jars placed in the autoclave in a metallic rack, the jars standing one above the other, the lower one being 4 or 5 inches above the bottom of the autoclave. These jars held approximately 10 pounds of soil. The soils were heated at 30 pounds pressure for three hours, at a temperature of about 135° C. The soils were treated with alkali just as soon as they had cooled to room temperature, so that only the changes caused by the immediate heating were under observation.

METHOD OF EXTRACTION.

Owing to the fact that only small quantities of organic matter are in these soils and also that not all of this organic matter can be extracted, large quantities of soil were used. Generally 40 to 50 pounds of soil, either heated or unheated, were extracted with 20 gallons of 2 per cent sodium hydroxide solution, the soil and solution being placed in a porcelain-lined, iron tilting kettle of a capacity of 30 gallons. This kettle is provided with a power-stirring device, with which the soil was stirred for seven hours. The stirrer was then removed and the soil allowed to settle overnight and sometimes for a longer time. The solution was then drawn off by means of a siphon. To the soil solution so obtained a suitable acid, depending on the ensuing procedure, was added until the solution was acid and the silt and so-called humus bodies were well flocculated. Providing the methods to be used would not be interfered with by the presence of large quantities of iron salts, the acid solution was then filtered through a filter press under pressure. If, however, iron salts would be objectionable, the solution was filtered through filter paper in the usual way. In either case after the filtered solution had been made exactly neutral with sodium hydroxide it was concentrated to a volume of 2 or 3 gallons in a vacuum pan, the temperature of the liquid not rising above 52° C. In this way large quantities of soil were easily and safely handled and a great deal of time saved.

No attempt was made to study the humus precipitate. The chief interest in the work centered about the nitrogen compounds, especially those rendered soluble by the heating of the soil. Such compounds would appear chiefly in the alkaline extract, and later in the acid filtrate from the humus precipitate.

The action of the weak alkaline solution is apparently to remove the resinous and similar material from the soil grains and to dissolve water-soluble material, as well as to form sodium salts with any of the acids which are present in the soil.

The acid filtrate, after neutralization and concentration, was used in the isolation of the various constituents that have been found in previous work, as well as any new compounds that might be encountered. A description of the methods by which the compounds were isolated from these soils will now be given.

METHODS OF ISOLATION AND IDENTIFICATION.

The purine bases and creatinine.—The original alkaline soil extract was made acid with sulphuric acid, and after filtering and concentrating was made quite alkaline with sodium hydroxide and the precipitate, consisting largely of the hydroxides of iron and aluminum, filtered from the solution. The bases were then precipitated in boiling solution with Fehling's solution and a little dextrose. The copper precipitate, after thorough washing, was suspended in water and decomposed with hydrogen sulphide, and, after the sulphides were removed by boiling and filtering, the solution was concentrated to about 150 c. c. Small portions of this solution were tested for the presence of the purine bases and for creatinine by the following color reactions: (1) The Xanthine reaction, which consists in the evaporation of the solution on the water bath with a little concentrated nitric acid, a yellow residue being obtained, which, on treatment with a drop of sodium hydroxide, gives a red color. On heating, this color changes to a purplish violet, but returns to red on cooling. This reaction is given by xanthine and guanine. (2) Weidel's reaction, which is given by xanthine and guanine, and consists in heating some of the solution with hydrochloric acid and nitric acid, or with hydrochloric acid and a chlorate, until gas ceases to be evolved, and then evaporating to dryness. When exposed to an atmosphere of ammonia this residue will turn pink if either of the bases is present. (3) Kossel's test, which is given by xanthine, hypoxanthine, adenine, and guanine, and consists in evaporating the solution with nitric acid and bromine water, when, on addition of a drop of sodium hydroxide, a red color is obtained. By these simple color reactions it is possible to tell in a general way which of the purine bases are present. Creatinine is indicated by (4) Jaffe's reaction, by which an orange color is developed in the solution on addition of sodium hydroxide

and picric acid. (5) Weyl's reaction, giving an orange red color which changes to yellow on standing, produced by the addition to the solution of sodium hydroxide and sodium nitro-prusside. (6) Salkowski's test, which consists in adding acetic acid to the solution resulting from Weyl's test and heating, when a green color changing to blue is obtained.

If the three color reactions were positive for creatinine in the solution tested, zinc chloride and sodium acetate were added to a portion of the solution and allowed to stand for several days, when the characteristic crystals of creatinine zinc chloride were formed. These were separated on a porous plate, washed with a little cold water, boiled with some freshly precipitated lead hydroxide, filtered, and the filtrate concentrated to a small volume; on standing several hours crystals having the characteristic form, solubility, and color reactions of creatinine were obtained.

The remaining portion of the solution containing the purine bases was made ammoniacal and the bases precipitated with ammoniacal silver nitrate. The precipitate was filtered off, washed well with cold water, boiled with nitric acid (sp. gr. 1.10), and the solution filtered hot. On standing, if hypoxanthine, adenine or guanine were present they separated out in the form of their silver nitrate salts, while the xanthine remained in solution. These crystals were filtered off, decomposed in boiling water with hydrogen sulphide, and after the sulphides were removed ammonia was added to the solution, when guanine, if present, separated out, and was filtered off. The filtrate, which in one case contained both hypoxanthine and adenine, was treated with picric acid, when the adenine, if present, was precipitated as adenine picrate. This was filtered off, washed with a little cold water, and taken up in a solution of dilute hydrochloric acid, and the solution shaken out with ether made alkaline with sodium hydroxide, and the adenine precipitated as the copper salt, with Fehling's solution and dextrose. After treatment of this precipitate with hydrogen sulphide the adenine was further identified by the formation of its various characteristic salts—the hydrochloride, the nitrate, the dichromate, the picrate, and the platinum chloride salts.

The filtrate from the adenine picrate was treated with dilute sulphuric acid, extracted with benzene to remove the picric acid, and then precipitated in ammoniacal solution with ammoniacal silver nitrate solution. This precipitate after washing was decomposed with hydrogen sulphide and after freeing from sulphides and evaporating the hypoxanthine was identified by the formation of the hydrochloride, the nitrate, the platinum chloride, and the silver picrate salts.

The xanthine portion of the nitric-acid treatment was made alkaline with ammonia, and the resulting precipitate after washing was decomposed with hydrogen sulphide and the xanthine identified by color reactions and by the hydrochloride, the nitrate, and the crystalline form of the base itself.

Since guanine was found for the first time in soils during the course of this investigation, the methods used for its isolation and identification will be given in detail in another part of this bulletin.

Histidine and arginine.—The alkaline extract of the soil was made acid with sulphuric acid and filtered. After concentration the solution was made acid again with the same acid to about 5 per cent and filtered hot. After cooling phosphotungstic acid was added until precipitation ceased, when the solution and precipitate were allowed to stand for several days. The precipitate was then filtered off, washed with dilute sulphuric acid, and dissolved in a solution of three parts acetone and four parts water. Such a solution dissolves the phosphotungstic salts of the hexone bases. The solution was filtered from any insoluble matter and to the filtrate barium hydroxide was added to alkaline reaction, filtered, made slightly acid with nitric acid, and the acetone removed by heating. To the cooled solution silver nitrate was added until a drop of the solution caused a solution of barium hydroxide to turn yellow. Barium hydroxide was then added until an alkaline reaction persisted and precipitation ceased. This precipitate, which contains histidine, was filtered off and washed with dilute barium hydroxide solution, suspended in dilute sulphuric acid and decomposed with hydrogen sulphide. After the removal of the sulphides by boiling and filtration the solution was made slightly alkaline with barium hydroxide to precipitate the sulphuric acid and the barium removed with carbon dioxide. The solution was boiled, filtered, and evaporated to dryness. The residue was taken up with a little silver nitrate solution to which a drop of nitric acid had been added, and was filtered. To the filtrate ammonia was added until the precipitation was completed and the precipitate filtered off, washed, and decomposed on the filter with dilute hydrochloric acid. The solution so obtained was evaporated to small volume, when histidine in the form of the dichloride salt of histidine crystallized out in characteristic form.

To the filtrate from the precipitation of the histidine with barium hydroxide and silver nitrate, solid barium hydroxide was added and the precipitate, if any formed, was filtered off, washed with strong barium hydroxide solution and after suspension in dilute sulphuric acid, was decomposed with hydrogen sulphide. After freeing the solution from sulphides, barium hydroxide was added to remove the sulphuric acid, the barium removed with carbon dioxide, and the solution boiled and filtered. A portion of this solution was

made neutral with nitric acid and evaporated to a small volume, when arginine, if present, crystallized out in the form of the neutral nitrate. To the remaining solution hydrochloric acid was added to neutrality, and on evaporation the hydrochloride of the base was obtained.

Cytosine.—For the isolation of this compound sulphuric acid was also used to acidify the alkaline extract from the soil. The concentrated neutral solution obtained from the vacuum pan was filtered, made acid with sulphuric acid, and an excess of mercuric sulphate solution added, and the whole allowed to stand for 24 hours. The precipitate which formed was filtered off, washed well, and after being suspended in water was decomposed with hydrogen sulphide. When the sulphides had been removed by boiling and filtration, the solution was evaporated to a small volume and allowed to stand for a time. A crystalline mass separated out, which was mixed with coloring matter and foreign material; consequently the solution was evaporated to dryness and extracted a number of times with absolute alcohol. The alcohol removed much of the coloring matter, and by repeated fractional crystallization from water a white compound was obtained which showed the characteristic crystalline structure and shiny appearance of cytosine. This compound was further identified by its solubility in water, alcohol, and ether, and the preparation of the platinum chloride, hydrochloride, picrate, and potassium-bismuth iodide salt.

Nucleic acid.—In this case acetic acid was used to acidify the alkaline soil extract, and the concentrated liquor was filtered hot. After cooling, a little more than an equal volume of 95 per cent alcohol was added to the solution and enough hydrochloric acid to make the solution acid to about 5 per cent. After standing several days the precipitate was filtered off, washed with water, and dissolved in dilute sodium hydroxide. The nucleic acid was again precipitated by the addition of sodium acetate, hydrochloric acid, and alcohol. The precipitate was filtered, washed, and dissolved in dilute ammonium hydroxide. To this solution lead acetate was added to complete precipitation, and the precipitate was filtered off and washed thoroughly with water. The precipitate was broken up, after suspension in a large volume of water, with sulphuric acid, care being taken to avoid any excess of acid. From this solution the nucleic acid was again precipitated with sodium acetate, hydrochloric acid, and alcohol, and the precipitate after thorough washing on the filter was dried. The compound so obtained was fairly light in color and was amorphous. A portion of it was boiled with concentrated hydrochloric acid and tested for pentoses with the phloroglucin and orcin tests, which showed their presence. Another portion was evaporated with magnesium nitrate, ignited, and the residue taken

up with hydrochloric acid. This solution gave tests for phosphates both with ammonium molybdate and with magnesia mixture. The remainder of the substance was boiled with hydrochloric acid, made alkaline with sodium hydroxide, and the purine bases present precipitated with Fehling's solution, the precipitate being treated as described for the isolation of the purine bases. From this solution the Xanthine test was obtained, but the bases were present in such small quantities that their individual identity could not be ascertained.

Dihydroxystearic acid.—To acidify the alkaline extract in this case sulphuric acid was used. To the concentrated solution obtained later, sulphuric acid was again added to strong acid reaction and the solution filtered hot. After cooling, the solution was shaken out twice with ether. The ether solution was quite strongly colored, and it was found that if this solution were shaken out with a saturated solution of sodium bisulphite the bisulphite solution absorbed almost all the color. The ether solution was then evaporated over a small quantity of water, about 100 c. c., and this solution boiled and filtered through a filter paper wet with hot water. On standing the dihydroxystearic acid, if present, crystallized out and on drying on a porous plate was white and melted at 99° C.

An unidentified aldehyde.—The bisulphite solution from the above extraction was made acid with sulphuric acid and the sulphur dioxide removed by heating on the steam bath. This solution on extraction with ether and evaporation of the ether over water gave an oily residue which was slightly soluble in water. The water solution gave a violet color when a dilute solution of ferric chloride was added to a portion of it, and also gave a slight test for aldehydes with the fuchsine aldehyde reagent. Attempts to make either a phenylhydrazine, hydroxylamine, or semicarbizid compound failed. The aldehyde acts somewhat like salicylic aldehyde, but the amount obtained was too small to enable identification.

Pentosans, pentose sugars, and xylose.—Acetic acid was used to acidify the alkaline soil extract, and after the solution had been filtered through paper it was made neutral with sodium hydroxide and again filtered to remove the precipitate of aluminum, etc. To this solution neutral lead acetate was added, the solution stirred several times, and the lead precipitate allowed to settle for several days. The clear solution above the precipitate was siphoned off, made quite alkaline with ammonia, and allowed to stand a day or two. The precipitate obtained was separated first by siphoning off the supernatant liquid and then by filtering. It was washed well with water, suspended in water, and decomposed with hydrogen sulphide. The resulting solution after removal of the sulphides was concentrated to a small volume and on cooling was added to three

volumes of alcohol. On standing some time the pentosans settled out as a gummy precipitate and were filtered off and washed. Some of the precipitate was boiled with hydrochloric acid and tested with phloroglucin and orcin. The remainder of the precipitate was boiled with dilute sulphuric acid, the acid removed with barium hydroxide, and the barium with carbon dioxide. To a portion of this solution cadmium carbonate and bromine water were added, and the resulting double salt of cadmium xylonate and cadmium bromide was obtained in characteristic crystalline form. An ozazone was also prepared, but could not be obtained pure enough for a melting point test.

Pentose sugars were determined in the filtrate from the pentosan precipitate by evaporating off the alcohol and making the two tests with hydrochloric acid, phloroglucin, and orcin. Attempts were made to isolate these sugars, but they were unsuccessful.

ISOLATION AND IDENTIFICATION OF GUANINE.

When ammonia was added to the solution containing hypoxanthine, adenine, and guanine, as already described, there was formed, in the case of the Sassafras heated soil, a gelatinous precipitate. This precipitate was filtered off, washed with a little cold water, and then dissolved in warm dilute hydrochloric acid. On evaporating a little of this solution to dryness with nitric acid and placing the dish containing the residue in an atmosphere of ammonia a pink color was developed. The solution remaining was divided into two portions. To the first portion, which had been previously warmed, a cold saturated solution of picric acid was added, when the picrate described by Capranica [1] separated out in crystalline form, which under the microscope appeared as pencil-shaped, fernlike tufts of orange yellow needles. They were very insoluble in water, and on drying took on a feltlike appearance with a silky luster, and became golden yellow in color. On heating they became orange red and began to decompose at 190° C.

To the other portion a solution of potassium dichromate was added, and on standing the dichromate salt described by Wulff [2] separated out in large, well-formed, bright orange colored, elongated, four-sided prisms with truncated ends. This salt was allowed to dry and was then heated to about 100° C., when it lost water and turned a dark violet color.

By the method of obtaining this base, its solubility in water, ammonium hydroxide, and hydrochloric acid; the solubility of the silver nitrate salt in nitric acid of specific gravity 1.10; the various color reactions, and the two characteristic salts, the picrate and the dichromate, the identity of the compound is established as guanine.

[1] Zeit. physiol. Chem., 4, 233 (1880).

[2] Ber. deutsch. chem. Ges., 30, 2241 (1897).

COMPOUNDS ISOLATED FROM THE HEATED AND THE UNHEATED SOILS.

The procedure followed in carrying out this work and isolating or identifying the organic constituents in the unheated and heated soils has been briefly described in the foregoing. The results of this part of the investigation are concisely given in the following table:

TABLE III.—*Organic compounds isolated from heated and unheated soils.*

Elkton silt loam.		Sassafras silt loam.	
Unheated.	Heated.	Unheated.	Heated.
Nucleic acid.	Nucleic acid.	Nucleic acid.	Nucleic acid.
Xanthine.	Xanthine.	———	Xanthine.
———	Hypoxanthine.	Hypoxanthine.	Hypoxanthine.
Adenine.	Adenine.	———	———
———	———	———	Guanine.
———	Cytosine.	———	Cytosine.
Pentose sugar.	Pentose sugar.	Pentose sugar.	Pentose sugar.
Pentosans.[1]	Pentosans.	Pentosans.	Pentosans.
Histidine.	Histidine.	Histidine.	Histidine.
———	———	———	Arginine.
Creatinine.	Creatinine.	Creatinine.	Creatinine.
Dihydroxystearic acid.	Dihydroxystearic acid.	———	Dihydroxystearic acid.
———	Unidentified aldehyde.	———	Unidentified aldehyde.

[1] From the pentosans of this soil xylose was obtained, showing that it was, at least in part, xylan.

In harmony with other investigators, the total amount of water soluble solids was increased by the heating. The soils when treated with four parts of water showed, by the bridge method, the following content in total solids: The normal Elkton soil, 184; the heated Elkton soil, 336; the normal Sassafras, 151; the heated Sassafras, 270 parts per million of dry soil. It will be noticed that the poorer soil showed the higher content of total solids under both heated and unheated conditions. No further examination of the saline material was made. By heating, the acidity of both soils was increased. It should be noted, however, that ammonia and amines were formed by the process of heating, as was indicated by the fishy odor upon adding the alkali in the process of extraction.

The results in Table III show only whether the organic compound was present or not. While no quantitative methods for the separation of these compounds from soils exist, owing to a great variety of difficulties which can not be entered into here, still in the work it was possible to distinguish between very small amounts and larger ones, and these differences are of interest and in a few instances highly instructive.

As seen from the table, the nucleic acid was found in both soils, in the heated as well as in the unheated samples. However, from the heated samples less nucleic acid was obtained than from the unheated samples, which fact is in harmony with the increase in nucleic acid decomposition products.

Xanthine was found in both heated and unheated Elkton soil, but in larger quantity in the heated soil. In the case of the Sassafras soil it was found only after heating.

Hypoxanthine was not found in the Elkton soil, but after heating a small quantity was obtained. The Sassafras soil contained this constituent, but its quantity was much increased by heating the soil.

Adenine was found in small quantity in the Elkton soil, but was not obtained from the Sassafras. Heating effected no change in either case.

Guanine was found only in the Sassafras soil after heating and is of especial interest, since this is the first time it has been encountered in soil.

Cytosine is likewise a decomposition product of nucleic acids, and it is interesting to note that it was found only in the heated samples of both soils. It was obtained in much larger quantities in the case of the heated Sassafras soil than in the heated Elkton soil.

Pentose sugar was indicated in both soils, heated and unheated. Pentose sugar reactions have been given, however, by every soil tested in this laboratory, which is also the case with pentosans.

Histidine occurs in both soils, and a slight increase was noticed as the effect of the heat.

Arginine, on the other hand, was found only in the heated Sassafras.

Creatinine was present in all cases.

The unidentified aldehyde in the above table, as has already been mentioned, is an aldehyde with a benzene ring. It was found in small quantities, but only in the heated soils, and may, therefore, be assumed to be formed during the process of heating.

Dihydroxystearic acid occurs in the Elkton soil, but not in the Sassafras soil, as has been reported in a special study of the distribution of this compound in soils, presented in a former bulletin.[1] The present samples of the two soils, from the same fields but collected two years later, showed this same relationship, indicating that the compound is a rather constant constituent of the Elkton soil under the field conditions to which it has been subject. The heated Elkton soil showed an increase in the quantity of dihydroxystearic acid. While no dihydroxystearic acid exists in the Sassafras soil under normal conditions, it is significant that after heating, a very considerable quantity was isolated—a quantity even larger than that found in the unheated Elkton soil and fully as large as in the heated Elkton soil.

CHEMICAL CHANGES INCIDENT TO THE HEATING OF SOILS.

While it is not the purpose to discuss here fully all the changes which are involved in protein decomposition, it is, however, necessary

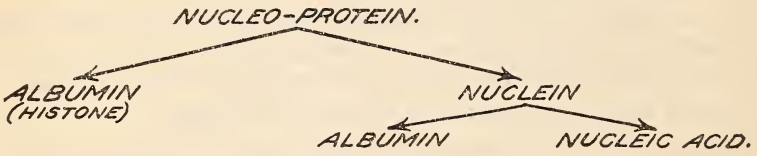
[1] Bul. 80, Bureau of Soils, U. S. Dept. Agr. (1911).

to consider certain phases of the subject related to this investigation, so that a clear conception of the changes observed in the course of this investigation may be obtained.

There get into the soil continually such complex organic compounds as proteins, lecithins, nucleic acids, nucleo-proteins. While the final products of decomposition of these are no doubt such simple combinations or elements as carbon dioxide, marsh gas, ammonia, nitrogen, hydrogen, these products are arrived at by certain definite and fairly well understood stages of degradation. Depending on the factors present under various soil conditions, this process may be a rapid one, a slow one, or one entirely arrested at certain stages. The latter condition may be due to the fact that some of the factors necessary to accomplish the next change in the process of degradation are absent.

NUCLEO-PROTEIN DEGRADATION.

Nucleo-proteins are perhaps the most complex of the compounds which enter the soil. They are common constituents of plants, animals, bacteria, and molds, and occur, therefore, wherever these live and die. It will be well to trace such a complex compound through the chemical changes which it suffers by decomposition. Lilienfeld [1] has expressed the decomposition of a nucleo-protein by the following schematic representation:



The products then are albumin or protein and nucleic acid. Protein material has not been isolated as such from soils, but that it is present is indicated by the production of its decomposition products, as well as by the occurrence of these products in soils under normal conditions. The isolation of nucleic acid from soils, on the other hand, has been accomplished.

NUCLEIC-ACID DEGRADATION.

The nucleic acid is still a very complex compound and on decomposition yields a large number of compounds of somewhat less complicated nature. The name nucleic acid is a class name, covering a large number of compounds composed of carbon, hydrogen, oxygen, nitrogen, and phosphorus. The acid occurs in plant and animal cells and has been isolated from soils.[2]

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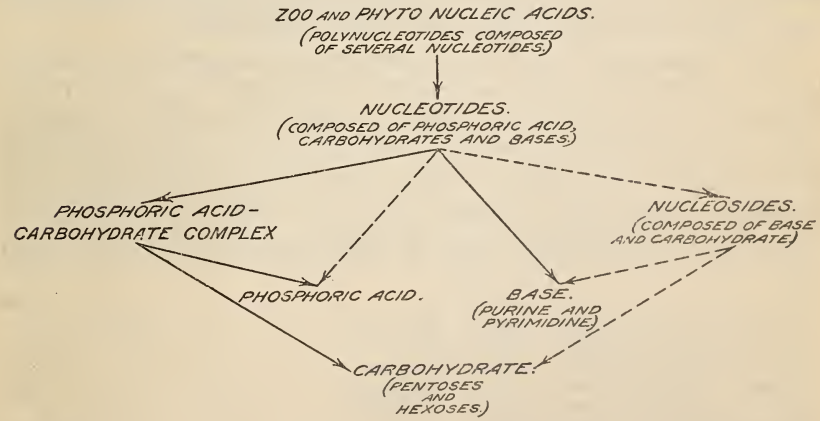
[1] Arch. f. (Anat. u.) Physiol., 1892, p. 128.

[2] Shorey, Science, 35, 390 (1911); Bul. 88, Bureau of Soils, U. S. Dept. of Agr., 1912.

The proportion of the above elements in nucleic acid differs, depending upon the source of the nucleic acid, and this is due to the fact that the unit substances composing it are different. Thus, for instance, the nucleic acid from yeast, according to Herlant,[1] has the composition $C_{36}H_{48}O_{30}N_{14}P_4$, that from salmon, according to Schmiedeberg,[2] the composition $C_{40}H_{52}O_{25}N_{14}P_4$, and that from wheat, according to Osborne and Harris,[3] $C_{41}H_{61}O_{31}N_{16}P_4$.

The constitution of these complex nucleic acids is unknown, but the constitution of most of the dissociation products is known. As above mentioned, these dissociation products vary according to the source of the nucleic acid. These degradation products are: Phosphoric acid, which is always found; carbohydrates, which are always found; laevulinic acid, which is sometimes found; pyrimidine derivatives, which are always found; and purine bases, which are also always found. According to which pyrimidine base, thymine, cytosine, or uracil is present, or according to which purine base, guanine, adenine, xanthine, or hypoxanthine is present, the composition of the acid varies. Since several of these compounds are present at the same time, a great variety of combinations is possible.

While the simpler end products of nucleic acid are as above mentioned, the dissolution of the nucleic acid molecule does not take place in one step. There are several intermediary products or combinations of the above-mentioned final products which have definite chemical and physical identity. The researches of Levene and his school have served to clear up to a large extent the nature of these intermediary products. The steps in the disintegration of the plant and animal nucleic acids can perhaps be best made clear by the following schematic representation:



The complex zoo or phyto nucleic acids, for instance those from wheat, yeast, salmon, are composed of simpler nucleic acids which Levene has called nucleotides. The nucleotides are composed of phosphoric acid, carbohydrates, and bases, either purine or pyrimidine.

[1] Arch. exp. Path., 44, 148 (1900). [2] Arch. exp. Path., 43, 57 (1899). [3] Zeit. physiol. Chem., 36, 85 (1902).

Several of these nucleotides have been isolated, for instance, inosinic acid,[1] guanylic acid,[2] cytidin-phosphoric acid,[3] uridin-phosphoric acid,[3] thymohexose-phosphoric acid.[4] On further decomposition the nucleotide can part with its phosphoric acid on the one hand and yield a complex consisting of the base and the carbodydrate, designated as nucleoside in the diagram, and on the other hand it can part with the base, and leave a complex consisting of phosphoric acid and the carbohydrate. Of the nucleosides, a considerable number are known, inosin,[4] guanosin,[4] adenosin,[4] xanthosin,[5] which are, respectively, hypoxanthine, guanine, adenine, and xanthine combined with the carbohydrate. Further, from the nucleotides, cytidin- and uridin-phosphoric acids, the nucleosides cytidin [5] and uridin [5] are obtained which consist, respectively, of combinations of cytosine and uracil with carbohydrate. Several of these compounds are well-defined crystalline bodies and form salts of the same type as the base contained in them. A definite carbohydrate-phosphoric acid complex has also been isolated and a crystalline barium salt obtained. This is the d-ribose phosphoric acid[6] obtained by Levene and Jacobs. All of these various products can then be broken down into the phosphoric acid, carbohydrate, and base, purine or pyrimidine.

TRANSFORMATION OF THE BASES.

The purine and also the pyrimidine derivatives are capable of being changed one into the other, by both chemical and biochemical agencies. Nitrous acid has been used to effect such changes. In this manner, Fischer[7] changed guanine into xanthine, Kossel[8] changed adenine into hypoxanthine, and Kossel and Steudel[9] changed cytosine into uracil. Similarly Levene[10] has changed the complexes guanosin, adenosin, and cytidin into xanthosin, inosin, and uridin, respectively.

These changes produced by strictly chemical means are paralleled by the biochemical changes induced by bacteria and enzymes. It was shown by Schittenhelm and Schröter [11] that putrefactive bacteria, especially coli bacillus, were able to convert adenine and guanine into hypoxanthine and xanthine. They also show that the bacteria have the power of splitting down the nucleic acid itself. This change of nucleic acid is also brought about by certain enzymes which have been called nucleases, such as erepsin.[12]

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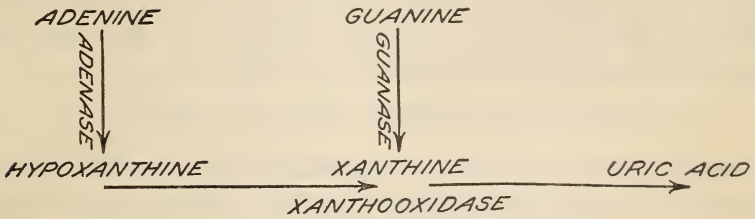
[1] Levene and Jacobs, Ber. deutsch. chem. Ges., **44**, 746 (1911).
[2] Levene and Jacobs, Biochem. Zeit., **28**, 127 (1910).
[3] Levene, Abderhaltens Biochem. Arbeitsm., V, 489 (1911).
[4] Levene, Abderhaltens Biochem. Arbeitsm., II, 605 (1910).
[5] Levene, Abderhalten, Biochem. Arbeitsm., V, 489 (1911).
[6] Ber. deutsch. chem. Ges., **41**, 2703 (1908).
[7] Ann. Chem. Pharm., **215**, 253 (1882).
[8] Zeit. physiol. Chem., **10**, 258 (1886).
[9] Zeit. physiol. Chem., **38**, 54 (1903).
[10] Abderhaldens, Biochem. Arbeitsm., V, 489 (1911).
[11] Zeit. physiol. Chem., **41**, 284 (1904).
[12] Nakayama, Zeit. physiol. Chem., **41**, 348 (1904); Iwanoff, Zeit. physiol. Chem., **39**, 31, (1903); Sachs, "Ist die Nulslease mit dem Trypsin identisch." Diss. Heidelberg, 1905.

Schittenhelm [1] has shown the presence of an amidase in the pressed juice of lupine seeds capable of converting guanine into xanthine.

Burion [2] showed that enzymes were capable of oxidizing xanthine and hypoxanthine into uric acid. The existence in animal tissues of amidases capable of causing the conversion of the amido-purines (guanine and adenine) into the oxypurines (xanthine and hypoxanthine) has been repeatedly demonstrated. These changes produced by the various ferments are perhaps best shown by the diagram given by Jones and Austrian.[3]



The above exposition of the changes involved in the degradation of nucleic acid makes clear the fact that this operation occurs in many stages. Also that some of these intermediary as well as final products are capable of being transformed one into the other. This degradation and transformation can be brought about in many different ways: By chemical means, such as the action of acids, alkalies, and steam under pressure; by microorganisms; and by enzymes. The fact that such changes have been worked out in other lines of research enables us to interpret the changes observed in soils when heated, as well as to explain differences observed between soils not heated.

CHEMICAL CHANGES IN THE NUCLEIC ACIDS IN THE SOILS.

Applying these principles to the interpretation of the results obtained by heating the Elkton and Sassafras soils, it becomes evident at once that hydrolysis of the nucleic acids in these soils has taken place. This is evidenced by the fact that in each case there was a diminution in the amount of nucleic acid obtained from the soils, and by the increase, both in quantity and in number, of the nucleic acid bases.

In the Elkton soil unheated, xanthine and adenine were found; on heating, the amount of xanthine increased, adenine was found as before, and in addition hypoxanthine appeared. The hypoxanthine has been formed from a nucleotide or a nucleoside containing this base. The fact that it did not appear in the unheated soil may be due to an arrested decomposition of its antecedents or to the fact that

[1] Zeit. physiol. Chem., 63, 289 (1909).
[2] Zeit. physiol, Chem., 43, 497 (1905).
[3] Jour. Biol. Chem., 3, 228 (1907).

it is changed by enzymes or microorganisms into xanthine or lower nitrogenous compounds. Similarly, the appearance of cytosine in the heated soil has undoubtedly arisen from the same cause, namely, the action of the heating on antecedents containing it.

In the Sassafras soil the changes are even more definite. In the unheated soil the only nucleic-acid base found was hypoxanthine, but after heating there appeared xanthine, guanine, and cytosine, as well as a larger quantity of the hypoxanthine. The absence of xanthine and guanine in the unheated soil would seem to indicate that their antecedents are not subject to further decomposition under these soil conditions or else that they are rapidly changed to uric acid, urea, ammonia, etc., under the existing soil conditions.

BIOCHEMICAL DIFFERENCES BETWEEN THE SOILS.

These two soils, as already mentioned, have had the same general field treatment and have received the same kind of organic matter in the form of manures and crop débris, so that the nucleo-proteins, nucleins, nucleic acids, etc., which have entered these soils have been practically the same. The different results in the heated as well as in the unheated soil can only show that the decompositions occurring normally under the two different soil environments are biochemically different. It has been repeatedly emphasized in the publications from this laboratory that the organic matter of different soils is different, and in the present instance not only is this clearly shown, but it is pointed out that this difference is brought about by biochemical factors.

One difference between the two soils is found in the fact that the Elkton soil contains no antecedent compound which will yield guanine by heating. This fact may be explained by the assumption that the guanine antecedent, possibly guanosin, is changed to a xanthine antecedent. Color is lent to this assumption by the fact that xanthine is found in both the unheated and heated soil. On the other hand, guanine as well as xanthine is found in the heated Sassafras soil, whereas neither is found in the unheated.

Another difference lies in the adenine. This is found in the Elkton soil and not in the Sassafras, and any antecedent bodies seem also absent from the latter soil, since it is not produced by heating. Hypoxanthine, on the other hand, into which adenine or its antecedents may change, is absent from Elkton but present in Sassafras.

Cytosine antecedents are present in both soils. The fact that cytosine itself is not found in the unheated soils may again be due to permanency of the antecedent under both soil conditions or else to the presence of factors, either chemical or biochemical, which transform it into uracil. Whether uracil exists in these soils or, in fact, any other soil can not be experimentally determined at present,

owing to lack of characteristic derivatives or properties of uracil, although it seems probable that it must exist at least under certain conditions.

PROTEIN DEGRADATION.

The nucleic acid decomposition has been followed in the preceding sections from its combination in a nucleo-protein to its end products. The albumins or proteins which split off in the early stages, as well as proteins which exist as such in plant and animal tissues, are, if anything, more complex than the nucleic acid, and the intermediate products between protein, on the one hand, and the final products of complete hydrolysis, on the other, are less clearly defined than in the case of the nucleic acid products. It is certain, however, that there are such products. The final products are well known and their properties clearly defined.

Two of these final products of protein hydrolysis appear in the present investigation. These are arginine and histidine. The latter was found in both soils, unheated and heated, but was found in greater quantity in the heated soil. Arginine, on the other hand, was found only in the heated Sassafras soil. There is present, therefore, in the Sassafras soil a protein complex which acts as the antecedent of arginine. The question then remains, Why is arginine absent from the unheated soil, and also why is this antecedent body absent from the Elkton soil? Arginine, although a very common primary dissociation product of proteins, has, nevertheless, only been found in two other soils. This is rather peculiar in view of the fact that its close associate, histidine, has been found very often, a fact which is best explained on the supposition that arginine is very susceptible to changes through biochemical influences. For instance, Kiesel[1] found that the arginine in the juice of the green sprouts of lupine disappeared in the course of a four weeks' autolysis. It seems probable that its susceptibility to change is the reason of its nonappearance in the normal Sassafras soil. In regard to the second part of the question, as to the absence of any direct antecedent body, no very definite statement can be made, but it seems reasonable that, since there is protein material still in the soil, the change has not proceeded to a stage where arginine could be produced by such a procedure as was here employed. In other words, the biological factors in the Sassafras soil were such as to furnish an intermediary product which was dissociable by heat. From this is would again appear that the biochemical factors in the two soils are somewhat dissimilar under field conditions.

Concerning creatinine, nothing definite is known as to its antecedent compounds and no special lesson can be learned from the occurrence of this constituent in these soils. The same may be said of pentose sugars and pentosans.

[1] Zeit. physiol. Chem., 60, 460 (1909).

BENEFICIAL EFFECTS OF THE ORGANIC NITROGENOUS COMPOUNDS FORMED.

The compounds considered thus far, taken as a whole, show an increase in the heated soil, both in quantity and in number. This is in harmony with the often observed analytical fact that the heating produces an increase in soluble nitrogenous material. These results show the nature of the compounds thus made soluble. It remains to discuss the effect of such compounds on plant growth. Nearly all of these soil constituents have been studied in regard to their effect on plant growth and the results reported in an earlier bulletin.[1] Among those tested were nucleic acid, hypoxanthine, xanthine, guanine, histidine, arginine, and creatinine. All of these were shown to be decidedly beneficial to the growth of wheat seedlings.

Take, for instance, hypoxanthine. The effect of this soil constituent was studied in solutions containing many different ratios of fertilizer elements. The effect of the hypoxanthine was very marked in those cultures containing phosphate and potash but no nitrate. The average increase in growth in these cultures as a result of the presence of 100 parts per million of hypoxanthine was 41 per cent. When nitrate was also present, the increase due to hypoxanthine was not so great, but is was shown that in the presence of hypoxanthine less nitrate was consumed by the plants. All the other compounds mentioned above behaved in this same general way. In the absence of nitrate, nucleic acid gave an increase of 74 per cent, histidine gave an increase of 30 per cent, arginine an increase of 33 per cent, and creatinine an increase of 36 per cent. Guanine and xanthine were not tested in this same comprehensive manner, but the results show conclusively that they, too, are beneficial.

In view of these facts the cause of the increased productiveness of heated soils would seem to be explained by an increase in beneficial, readily and directly assimilable organic soil constituents. But what of the undoubted fact that some soils when heated show a harmful rather than beneficial effect on plant growth, especially on germination and the early stages of growth?

HARMFUL EFFECTS OF HEATED SOIL.

The heated and unheated soils, as well as water extracts from them, were tested for their effects on plant growth. The soil test was made in paraffined wire pots in the usual manner. The wheat seedlings grew from July 6 to July 21, 1911. The results of this test were as shown in Table IV.

[1] "Nitrogenous Soil Constituents and Their Bearing on Soil Fertility," by Oswald Schreiner and J. J. Skinner, Bul. No. 87, Bureau of Soils, U. S. Dept. of Agr. (1912).

TABLE IV.—*Plant growth on heated and unheated soils.*

Soil and treatment.	Green weight.	Relative growth.
	Grams.	
Sassafras silt loam	2.525	100
Sassafras silt loam, heated	1.070	42
Elkton silt loam	2.043	81
Elkton silt loam, heated	1.820	72

In harmony with the field experience on these soils, the Sassafras is the better soil, the Elkton giving in this case only 81 per cent of the growth obtained in the Sassafras soil. The heating, however, had a bad effect on both soils, by far the greater depression occurring in the normally better soil. All of this is of course surprising in view of the fact that more nitrogen compounds, shown to be in themselves beneficial, are liberated by heat in both soils, and particularly in the Sassafras soil. It is obvious that the chemical changes so far discussed do not explain this observed fact in regard to plant growth, and it is further obvious that some harmful compounds are produced in both cases, and apparently to the greatest extent in the Sassafras soil, in addition to the beneficial compounds already discussed. The depressed plant growth is also shown by the water extract from such heated soils as compared with the unheated, although, as might be expected, the effect is not so marked. The proportion of water and soil used was 4 of water in 1 of soil, and the solution culture made in the manner usual in this laboratory. The wheat seedlings grew from April 9 to April 19, 1912. The results are given in Table V.

TABLE V.—*Plant growth on extracts from heated and unheated soils.*

Soil extract.	Green weight.	Relative growth.
	Grams.	
Sassafras silt loam	5.100	100
Sassafras silt loam, heated	4.200	82
Elkton silt loam	4.800	94
Elkton silt loam, heated	4.600	90

The effects of the soil extracts are in the same order as the effects of the soils in the preceding table, the Sassafras heated being again the poorest medium for plant growth.

PRESENCE OF DIHYDROXYSTEARIC ACID IN THE ELKTON SOIL.

The lower productivity of the Elkton soil in the field and in laboratory experiments has been repeatedly noticed during several investigations conducted by this bureau. According to the latest publication [1] on this subject, this lower productivity may be explained,

[1] Bul. 80, already cited.

in part at least, by the presence in this soil of dihydroxystearic acid. This compound has been isolated from a number of unproductive soils, and its relation to infertility has thus been strongly indicated. Experiments with the compound itself have shown it to be very toxic to wheat seedlings, causing a diminution in growth, stunted root development with darkened root tips, often bent into hook-like forms, and diminishing very markedly the absorption of nutrients by the plants.

As before, the dihydroxystearic acid was found in this sample of Elkton soil and absent from the Sassafras soil. The dihydroxystearic acid is fairly easily destroyed or altered by oxidation. The influence of nitrate, which stimulates root oxidation,[1] and lime, which has the same effect on root oxidation, and probably also in the soil itself, since organic matter always disappears under its influence, have been shown to diminish the toxicity of the dihydroxystearic acid.[2] This remedial effect of lime and nitrate is also shown in the following cultural test, where several fertilizer elements were used, the rate per acre being 50 pounds of P_2O_5, K_2O, and NH_3. The lime was at the rate of 1 ton per acre.

TABLE VI.—*Effect of fertilizers on Elkton and Sassafras soils.*

Treatment.	Elkton.		Sassafras.	
	Green weight.	Relative growth.	Green weight.	Relative growth.
	Grams.		*Grams.*	
Check, nothing added	2.043	100	2.525	100
Phosphate	2.120	104	3.530	139
Potash	3.200	157	3.390	134
Nitrate	3.310	162	2.960	117
Phosphate, potash, and nitrate	3.225	158	3.790	150
Lime	3.272	160	2.240	88
Lime and nitrate	3.700	181	3.250	128
Lime and phosphate, potash, and nitrate	3.700	181	3.250	128

It is not the purpose here to discuss fully all the details of this test, but simply to call attention to the different relative responses of the two soils in regard to the fertilizers and lime. Both soils were acid in their reaction to litmus paper. The Elkton responds readily to lime, the Sassafras does not. The Elkton does not respond to acid phosphate; the Sassafras shows next to the largest increase produced, the largest being the complete fertilizer. In the case of the Elkton the larger increases were those in which nitrate and particularly nitrate and lime were used, which was not the case with the other soil. These facts are most strikingly shown by a comparison of the relative growth figures in the table.

[1] The Rôle of Oxidation in Soil Fertility, by Oswald Schreiner and Howard S. Reed, Bul. 56, Bureau of Soils, U. S. Dept. Agr. (1909).

[2] Some Effects of a Harmful Organic Soil Constituent, by Oswald Schreiner and J. J. Skinner, Bul. 70, Bureau of Soils, U. S. Dept. Agr. (1910).

INFLUENCE OF HEATING THE SOIL ON DIHYDROXYSTEARIC ACID.

Since this compound is apparently easily attacked, it was thought that heating the soil might cause a diminution of the quantity present, or perhaps the entire disappearance of the compound. It soon became evident, however, that on heating the Elkton soil the quantity of this compound increased rather than decreased. The plant tests in the water solution bore out this fact of an increase in the harmful constituent. The water solution of the heated Sassafras soil showed even greater toxicity than the heated or unheated Elkton soil.

This, as stated above, points to the generation of a toxic compound by the process of heating. Dihydroxystearic acid appears in the normal Elkton soil, which, as mentioned, has had the same sort of organic matter introduced into it as had the Sassafras soil, but which has been subject to different biochemical changes. The toxic substances generated by heat in the Sassafras soil should bear some relation chemically to the one found normally in the Elkton soil. Examination of the heated Sassafras soil showed the toxic compound to be identical with that in the Elkton soil, namely, dihydroxystearic acid; furthermore, this compound was obtained in even larger amount from the heated Sassafras than was obtained from the Elkton soil, either heated or unheated.

The production of this harmful compound, although this may not be the only one produced on heating, explains in itself the decrease in the fertility of the heated soil.

What the antecedent body in the Sassafras soil under field conditions may be is not known. Certain it is, however, that dihydroxystearic acid, as such, is not found in the Sassafras soil until after heating. The antecedent is probably the same in both soils, but under the influence of the different biochemical factors existing in the Elkton soil this antecedent compound is so changed that the harmful influence of the dihydroxystearic acid shows itself.

BALANCE BETWEEN BENEFICIAL AND HARMFUL FACTORS IN SOILS.

In the foregoing it has been shown that beneficial compounds have been increased by heating, but simultaneously with this increase there has been a production of harmful compounds. The effect on plant growth is naturally the mean of these two influences, and the full beneficial effects can not show themselves until the harmful compound has been eliminated by oxidation, reduction, cultural treatment, or other means. Similarly the full harmful effect of a compound is not attained in the presence of beneficial compounds. This state of affairs is, however, not an unusual one confined to heated soils, but is found in soils under normal field con-

ditions. The soils under consideration are an excellent illustration of this point. The Elkton soil contains beneficial constituents, but also contains dihydroxystearic acid, a harmful constituent. The plant growth upon it depends upon the balance of these two opposing factors; when one is decreased the other gains the ascendency. In the other soil—that is, the Sassafras—this harmful compound is absent or at a minimum under field conditions, and the beneficial compounds exert their full effect. Any change in soil condition may bring about a change in this balance of the opposing forces, so that the soil becomes temporarily or permanently more fertile or less fertile, as the case may be. The heating of the soil brings about a very rapid or sudden change in this respect, with the result that, even though beneficial compounds are liberated in this fertile soil, the production of the harmful compound more than overbalances them, with the result that the plant growth drops to 42 per cent of the normal. In the case of the Elkton soil, which already contained this compound, the effect of the heating was also to increase the beneficial compounds and only slightly increase the harmful compound, so that the net result was that crop growth was only depressed to 89 per cent of the normal. In other words, the balance was more disturbed in the case of the Sassafras soil than in the case of the Elkton. The effect of addition of fertilizers to the two soils was to alter this balance, and to alter it most in the case of the Elkton soil. Especially is this true in regard to the effect of lime and of nitrates in the two soils. Cultural methods or crop rotation can in the same way influence the biochemical factors in soils, and through them this balance of good and harmful properties.

When the dihydroxystearic acid has disappeared from the heated soil, through oxidation, cropping, liming, or use of nitrates, the full effect of the increased nitrogenous nutrients will manifest themselves and plant growth will be much better on the heated than on the unheated soil.

SUMMARY.

This bulletin deals with the chemical changes involved when soils are steam heated, as in the process of sterilization. The results may be summarized as follows:

(1) In accord with the work of other investigators it was found that there was an increase in water-soluble constituents and an increase in acidity. At the same time ammonia and amines were formed.

(2) By the process of heating there was an increase in all of the constituents isolated from the unheated soil except nucleic acid.

(3) By the process of heating there were formed xanthine, hypoxanthine, guanine, cytosine, and arginine, when not previously

existing. These compounds are decomposition products of nucleic acid and protein material and are all beneficial to plant growth.

(4) Guanine is reported for the first time as a constituent of soil organic matter.

(5) Dihydroxystearic acid was increased when present, and produced, when not previously present, by the heating process. This compound is harmful to plant growth.

(6) Both beneficial and harmful compounds were produced by heating the soils and were isolated. This bears out the experience of previous investigators with cultural tests.

(7) Cultural tests in these soils and their extracts showed that the heated soils gave a poorer plant growth.

(8) Although the majority of compounds formed must be classed as beneficial, the harmful compound formed at the same time more than overbalances their effects. Not until this harmful compound is eliminated or diminished can the full beneficial effects of heating be demonstrated.

(9) In soils there is a balance of beneficial and harmful factors, soil fertility or infertility being the resultant of the two groups. As one or the other group of factors gains the ascendency, the fertility is raised or lowered, as the case may be. This balance is influenced by cultural treatment, fertilizers, liming, crop growth, or crop rotation, etc., as well as by steaming.

(10) The results show that although the soils studied have received the same kind of organic matter and have received the same farm treatment, they have been subject to different biochemical factors, resulting in differences in their organic matter and in differences in their fertility.

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